Case Report

Diagnostic Value of Albumin Gene Expression in Liver Tumors: Case Report and Review of the Literature

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Management of a solitary liver mass necessitates reliable distinction between primary hepatocellular carcinoma and metastatic lesions. The histologic differentiation can be difficult even with special stains such as α-fetoprotein, cytokeratin, and carcinoembryonic antigen. Albumin is a specific product of hepatocytes, and in situ hybridization to reveal albumin messenger RNA (mRNA) is highly specific and sensitive for the diagnosis of primary hepatocellular carcinoma. This technique can be used on histopathologic specimens and in cytologic diagnosis. Herein we describe a patient with synchronous renal and hepatic masses, in whom the distinction had to be made between metastatic renal cell carcinoma and two separate primary tumors—one in the liver and one in the kidney. In situ hybridization for albumin mRNA proved helpful in making this distinction. In addition, we review the literature on the diagnostic use of albumin gene expression in liver tumors.

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AFP = α-fetoprotein; ISH = in situ hybridization; mRNA = messenger RNA

In the evaluation of solitary hepatic masses, distinguishing between primary hepatocellular carcinoma and other metastatic lesions is often necessary. Occasionally, such histopathologic differentiation is difficult[1,2] and becomes increasingly problematic with the widespread use of fine-needle aspiration biopsy specimens that necessitate cytologic diagnosis.[3-5] Although immunohistochemical stains for α-fetoprotein (AFP), cytokeratins, and other hepatocellular products are useful, these markers are not specific for hepatocellular lineage.[4,6] Furthermore, some hepatocellular carcinomas do not stain for AFP.[7]

Albumin is synthesized exclusively by hepatocytes and secreted into the bloodstream.[8,9] Some investigators have used immunohistochemical stains for albumin as a hepatocyte marker;[7] however, because of diffusion artifacts, immunohistochemical demonstration of albumin is unreliable.[1,4,10] In situ hybridization (ISH) to detect albumin messenger RNA (mRNA) has been shown to have remarkable specificity for hepatocellular lineage.[1,10] This technique can be used on histopathologic specimens and in cytologic diagnosis.[1] In this article, we describe a patient in whom this technique was applied and review the literature on its use in the diagnostic evaluation of liver tumors.

REPORT OF CASE

A 69-year-old man came to Mayo Clinic Rochester in July 1996 for a second opinion on management of metastatic renal cell carcinoma. Ten years earlier, he had undergone a left radical nephrectomy because of stage I renal cell carcinoma. He was free of disease until March 1996, when he sought medical attention from his local physician for management of abdominal pain. A computed tomographic scan of the abdomen revealed a 3-cm mass in the medial aspect of the right kidney and a 6-cm mass in the caudate lobe of the liver. Multiple benign renal cysts were also detected. These findings were confirmed by magnetic resonance imaging of the abdomen. A computed tomography-guided biopsy of the liver mass revealed low-grade adenocarcinoma, consistent with a primary renal lesion. Findings on upper gastrointestinal endoscopy and colonoscopy were normal. The patient was in good health and had no symptoms related to either the liver or the kidney lesion.

Initial assessment of the patient at the Mayo Clinic revealed an increased AFP level of 264 ng/mL (normal, less than 15), a finding that raised the suspicion of primary hepatocellular carcinoma. The patient, however, had no risk factors for hepatocellular carcinoma. We believed that the patient had either metastatic renal cell carcinoma of the liver or two separate primary lesions. Because the prognosis of metastatic renal cell carcinoma is dismal and the best chance for long-term survival relied on the finding of two separate primary lesions, we performed surgical resection of the liver and kidney lesions. The patient underwent a partial right nephrectomy with enucleation of the tumor. The liver mass was removed by segmentectomy.

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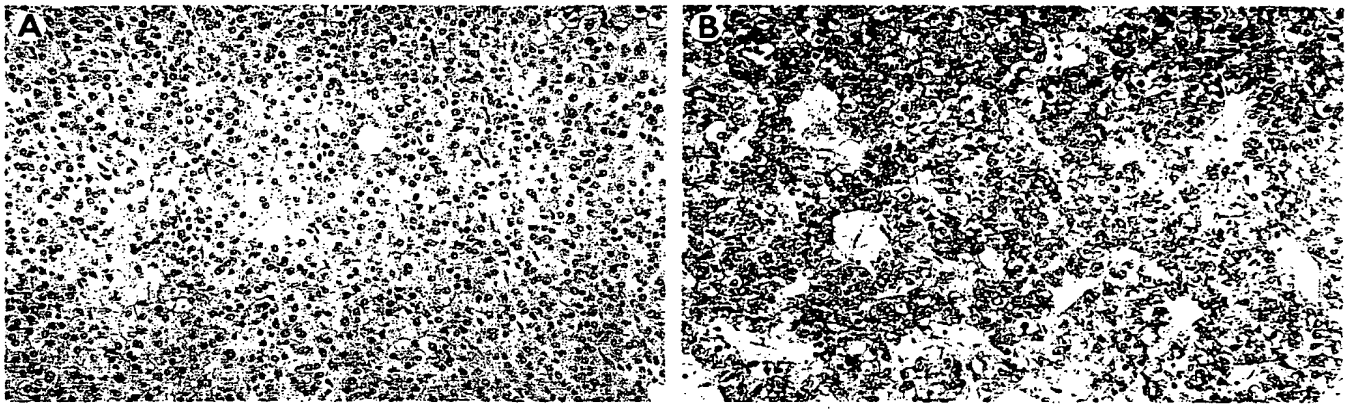

Fig. 1. Hepatocellular carcinoma. In situ hybridization for α-fetoprotein is negative (*A*) but strongly positive for albumin messenger RNA, as indicated by (blue) cytoplasmic staining (*B*). (Original magnification, x50.)

The patient had an uneventful recovery. After 1 year of follow-up, he has had no evidence of recurrence of either tumor. His follow-up serum AFP level, determined on two occasions since his operation, has been 1.4 ng/mL.

PATHOLOGIC FINDINGS

Pathologic examination revealed a grade 1 renal cell carcinoma that formed a variegated mass (4 by 3 by 2.7 cm) in the right kidney. The liver lesion consisted of a multilobulated mass (7 by 6.2 by 5.3 cm) that was consistent with a moderately differentiated adenocarcinoma. All surgical margins were free of tumor. The liver lesion stained negative for AFP (Fig. 1).

Cytokeratin Staining

After deparaffinization and rehydration of tissue specimens, slides were loaded onto a Ventana ES Autostainer (Tucson, Arizona). Pretreatment with protease was performed, and the slides were then stained by using cytokeratin AE1/AE3 antibody (Boehringer Mannheim, Indianapolis, Indiana) at a dilution of 1 to 400. Antibody binding was demonstrated with use of the labeled streptavidin-biotin method and with AEC (3-amino-9-ethylcarbazole) chromagen. The liver tumor was negative for cytokeratin with use of these antibodies. The renal tumor was cytokeratin positive by using the same antibodies and was clearly a primary renal cell carcinoma. Nevertheless, distinguishing whether the liver lesion represented a metastatic renal cell carcinoma or a synchronous primary hepatocellular carcinoma was still not possible.

ISH for Albumin mRNA

ISH for albumin mRNA was done on the resected liver specimen and was strongly positive for and diagnostic of hepatocellular carcinoma (Fig. 1). ISH was done on the renal tumor and was negative for albumin mRNA. Therefore, we determined that the patient had two separate primary tumors, rather than metastatic renal cell carcinoma. The details of the ISH technique have been previously described;[1,10,11] a brief description is provided below.

Sections of 4 μm in thickness were obtained from blocks containing nonfrozen tissue and having representative areas of the lesion. ISH was performed on the sections and on appropriate negative and positive controls. The oligonucleotide probes for albumin were synthesized in the Department of Biochemistry and Molecular Biology at the Mayo Clinic with use of an automatic synthesizer. Five oligonucleotides were made from the published albumin copy DNA sequence.[12] These included the regions of the copy DNA coding for amino acids −17 to −8, −2 to 8, 111 to 121, 291 to 300, and 561 to 570. The oligonucleotide probes were compared to GenBank sequence libraries in order to ensure specificity. Probe purification, labeling, and ISH were performed as previously described.[11] For enhancement of probe hybridization, tissue sections were microwaved in a 10-mM citric acid solution for 20 minutes before prehybridization. The positive control consisted of normal liver tissue. The negative controls for ISH consisted of pretreatment of the slides with ribonuclease A (250 μg/mL) before hybridization.

DISCUSSION

ISH to detect albumin mRNA in liver tumors is useful in distinguishing between primary hepatocellular carcinoma and lesions metastatic to the liver. The technique can be used on histopathologic[10] and cytologic specimens.[1] This technique is not useful for distinguishing between benign

liver lesions and hepatocellular carcinoma, but it serves to establish hepatic lineage because albumin is made exclusively in the liver. With use of ISH, albumin mRNA can be detected in formalin-fixed and paraffin-embedded tissue blocks, as well as in fresh specimens.[10] Thus, it is useful for routine pathologic diagnosis and retrospective research studies. Because albumin is made exclusively by hepatocytes, this technique is far more specific for hepatic lineage than are other markers, such as AFP. Thus far, a positive ISH for albumin mRNA has not been reported in tumors with "hepatoid differentiation" (for example, tumors in the gastrointestinal tract).

ISH was useful in making the diagnosis in our patient who had two synchronous primary tumors. His increased serum AFP level preoperatively raised the suspicion of hepatocellular carcinoma. Several tumors, however, including gastric and renal cancer, have been associated with an increased AFP level; thus, this finding is unreliable.[13,14] Although both the liver and the kidney lesions were resected in our patient, determining whether he had metastatic renal cell carcinoma with a solitary lesion metastatic to the liver or two synchronous primary tumors was still important because of associated prognosis. By performing ISH for albumin mRNA on the liver mass, the nature of that mass was determined to be primary hepatocellular carcinoma.

The accuracy of this technique has been assessed in both histologic and cytologic diagnoses. Yamaguchi and colleagues[10] studied the usefulness of ISH for the detection of albumin mRNA in 84 patients assessed at the University of Pittsburgh Medical Center. Both fresh specimens and paraffin-embedded tissue block specimens were used. Of the 59 patients with hepatocellular carcinoma, ISH was positive for albumin mRNA in specimens from 56 but negative in specimens from all 25 patients with known tumors metastatic to the liver or with cholangiocarcinoma. This corresponds to a sensitivity of 95% and a specificity of 100%. The investigators concluded that this technique was valid in the differential diagnosis of hepatocellular carcinoma from cholangiocarcinoma and tumors metastatic to the liver.

Papotti and coworkers[1] studied the usefulness of this technique in cytologic diagnosis of 97 fine-needle aspiration biopsy specimens from the liver. Their study included patients with known hepatocellular carcinoma and those with known metastatic carcinoma of the liver, as well as patients in whom the diagnosis was unclear. ISH was positive in 42 of the 44 patients known to have a primary hepatocellular carcinoma but was negative in the 20 patients with known tumors metastatic to the liver. These investigators reported a sensitivity of 95.5% and a specificity of 100%. This study demonstrates the usefulness of this technique in cytologic diagnosis of liver masses by fine-needle aspiration biopsy.

Factors such as age, sex, size of tumor, and presence of cirrhosis do not seem to affect the expression of albumin mRNA, and the grade of the tumor does not seem to affect the accuracy of this technique. Presence of albumin mRNA was detectable even in the most anaplastic cases of hepatocellular carcinoma. Nevertheless, a correlation between the grade of the tumor and the degree of albumin mRNA expression has not been demonstrated.[1,10] Investigators have noted that, within the same tumor mass, variable degrees of expression occur to the point that some areas may even lack albumin mRNA expression.[1] Therefore, tissue obtained from a needle biopsy may show no evidence of mRNA expression because of a sampling error, which results in a false-negative diagnosis. This is likely infrequent because the reported sensitivity of this technique exceeds 95%, even when fine-needle biopsy specimens are considered.

The occurrence of synchronous primary carcinomas in the liver and kidney in the same patient is extremely rare. We have not done studies to determine whether our patient had any genetic abnormality that would explain this manifestation.

CONCLUSION

ISH for albumin mRNA is a reliable and useful technique in the differential diagnosis of hepatic tumors. The test provides evidence to support hepatic lineage with a high degree of specificity, even in anaplastic lesions. It can be used on fresh specimens as well as on paraffin-embedded and formalin-fixed specimens. Furthermore, it can be used in histopathologic and cytologic diagnosis, a fact that enhances its utility in light of the increasing use of fine-needle aspiration biopsies. ISH for albumin mRNA will complement immunostaining for markers such as carcinoembryonic antigen and AE1 cytokeratin (which are negative in primary hepatic tumors and positive in metastatic lesions); thus, an accurate diagnosis can be made.

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Stamp Vignette on Medical Science

Erwin Neher— Nobel Laureate for Studies of Cell Function

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The 1991 Nobel Prize for medicine or physiology was awarded to biophysicist Erwin Neher (1944-) and physician Bert Sakmann (1942-) for their discoveries on the function of single-ion channels in cells. The two researchers devised a tiny electrode (a patch-clamp electrode) that could be used to identify the activity of ion channels (a type of membrane protein) that facilitate ion flow across a cell membrane. In medicine, ion channel studies have advanced the understanding of conditions such as cystic fibrosis, diabetes mellitus, epilepsy, and neuromuscular disorders.

Neher was born on Mar. 20, 1944, in Landsberg, Bavaria, Germany (about 20 miles south of Augsburg). After secondary schooling in his native region, he attended the Technical University in Munich, from which he received a bachelor's degree in physics. He left Germany to do graduate work at the University of Wisconsin in Madison and received an M.S. degree in 1967. From 1968 to 1972, he undertook graduate and postdoctoral studies at the Max Planck Institute for Psychiatry in Munich and earned his Ph.D. degree from the Technical University of Munich in 1970. During work on his doctoral thesis, he developed the idea of the patch-clamp technique.

In 1972, Neher transferred to the Max Planck Institute for Biophysical Chemistry in Göttingen (Germany). Two years later (1974), he began his collaborative research with Sakmann, which continued despite Neher's move to the University of Washington in Seattle and later to Yale University in New Haven, Connecticut. In 1976, Neher returned to the Max Planck Institute in Göttingen. He was named director of the Membrane Biophysics Department in 1983.

Neher was honored as a Nobel laureate on stamps issued in 1995 by both Grenada and Guyana.